PRESERVATION OF ANTISHEEP HEMOLYTIC AMBOCEPTOR IN GLYCEROL *

RALPH OAKLEY CLOCK AND STANLEY DREW BEARD

From the Lederle Antitoxin Laboratories, Pearl River, New York

In the complement-fixation test for the diagnosis of syphilis, gonorrhea, whooping cough, etc., it is usual to employ a hemolytic amboceptor which, although kept in a sterile manner, slowly deteriorates after several months even when stored in the ice-box.

The practical advantage of a suitably preserved and stable hemolytic amboceptor lies in the fact that it does not require retitration or standardization at different periods. There is also the advantage of the prevention of contamination, thereby prolonging the usefulness of the amboceptor.

To produce so stable an amboceptor necessitated the use of some suitable agent which would inhibit bacterial growth and chemical changes, thus preventing the immune bodies in the serum from deteriorating, and which, at the same time, would in no way interfere with the test to be conducted.

Tricresol, phenol, and chloroform were tried, but were discarded because they caused a precipitate when added to the serum in sufficient quantities to destroy bacteria, and they exerted no preservative power on the immune bodies contained in the serum. Furthermore, serum preserved with any of these chemical agents, after an indefinite period, often becomes anticomplementary so that it cannot longer be used.

The bactericidal properties of glycerol have been demonstrated by its use in the preparation of glycerolated vaccine virus; while its power to prevent molecular changes (due to its hygroscopic properties), thereby preventing deterioration, has been proved by its effective use in holding the standard diphtheria antitoxin, sent out by the United States government, unaltered for long periods. It was, therefore, decided to try glycerol as a preservative for antisheep hemolytic amboceptor. This investigation was begun on Feb. 7, 1914. Subse-

* Received for publication June 1, 1917.

quently, Ruediger[1] reported glycerol to be an ideal preservative for human serum intended for the Wassermann reaction. He also found that phenol, tricresol, and chloroform were not suitable for this purpose.

PREPARATION OF THE SERUM

Rabbits—large Belgian hares give the best result—immunized against washed sheep erythrocytes were anesthetized and bled from the carotid artery. The blood was collected in a sterile manner in small test tubes. As soon as the blood began to clot, the tubes were placed in the incubator for 1 or 2 hours. The clots were loosened from the wall of the tubes by means of a sterile wire, and the tubes then placed in the ice-box at 5 C. for 12-18 hours. The clear serum was pipetted off and inactivated at 55 C. for one-half hour. It was then mixed with an equal volume of sterilized, neutral glycerol and stored in small rubber-stoppered bottles. Repeated bacteriologic examinations carried out for 3 years have shown the sera preserved in this manner to be sterile at all times.

TITRATIONS

Antisheep hemolytic amboceptors, thus prepared, were titrated at intervals of 1-2 months during 3 years, using one tenth of the volume of the classic Wassermann reaction. As controls, similar titrations were made of serums preserved with other chemical agents, as well as serum without preservative.

TABLE 1

TITRATION OF GLYCEROLATED AND OTHER SERUMS

Amboceptor No.	Preservative	Date of Preparation	Date of Titration	Titer	Date of Titration	Titer	Date of Titration	Titer
121	0.3% Tricresol	7/ 7/13	7/22/13	1:2000	2/25/14	1:1000	2/23/17	Anticomplementary
123	0.5% Phenol	8/14/13	8/15/13	1:4000	2/25/14	1:1000	2/23/17	Anticomplementary
124	0.3% Chloroform	9/27/13	9/29/13	1:4000	2/25/14	1:1000	2/23/17	Anticomplementary
132A	None	2/ 7/14	2/25/14	1:5000	6/15/14	1:3000	2/23/17	Contaminated and anticomplementary
132B	50% Glycerol*	2/ 7/14	2/25/14	1:5000	6/15/14	1:5000	2/23/17	1:5000
133A	50% Glycerol after which the mixture was heated to 55 C. for one-half hour	2/ 7/14	2/25/14	Anticomplementary	6/15/14	Anticomplementary	2/23/17	Anticomplementary
133B	50% Glycerol*	2/ 7/14	2/25/14	1:2000	6/15/14	1:2000	2/23/17	1:2000
141	50% Glycerol*	2/16/14	2/25/14	1:3000	6/15/14	1:3000	/2 23/17	1:3000

* In the preparation of these amboceptors, glycerol was added after the serums had been inactivated at 55 C. for one-half hour.

[1] Philippine Jour. Sc., Sect. B, 1916, 11, p. 1.

Serums 132 and 133 were divided into 2 portions, A and B. No preservative was used in 132 A, but 132 B was preserved in glycerol as described. Serum 133 A was mixed with an equal volume of glycerol, and then heated to 55 C. for one-half hour; while the other portion, 133 B, was preserved in glycerol, according to the method described.

As will be seen from Table 1, only the serums which were preserved in 50% glycerol retained their original titer after 3 years; and further, such serums did not develop anticomplementary properties nor become contaminated.

ANTICOMPLEMENTARY PROPERTIES

If the serum was first mixed with glycerol, and the glycerolated serum then heated to 55 C. for one-half hour (as was done in the case of 133 A), the serum was rendered anticomplementary. The anticomplementary properties thus produced could not be destroyed by repeated heating at any time during the 3-year period. As pointed out by Ruediger,[2] in order to prevent glycerolated serum from becoming anticomplementary, the serum must be inactivated at 55 C. for one-half hour before being mixed with glycerol.

COMPLEMENT-FIXATION TESTS

Antibody-content titrations were made with several antigens and known positive sera, using 141 and 132 B amboceptors (preserved in glycerol) in order to determine what effect, if any, the glycerolated amboceptor had on the reaction. In order to have adequate controls, duplicate tests were made with amboceptor 132 A and an amboceptor (250) obtained from the New York City Department of Health, which latter contained no preservative; in this respect 132 A and 250 were similar except that they were prepared in different laboratories.

Neutral extracts of pure cultures of gonococci, meningococci, and streptococci were used as antigens; also saline extracts of pollen. One fourth of the anticomplementary dose was used in each tube.

The serums of guinea-pigs were used in a 10% dilution of physiologic salt solution as complement.

A 5% suspension in physiologic salt solution of washed, sedimented sheep corpuscles was used.

[2] Philippine Jour. Sc., Sect. B, 1916, 11, No. 2, p. 87.

TABLE 2

RESULTS OF COMPLEMENT-FIXATION TESTS*

Antigen and Dilution	Amboceptor	Amount of Antigonococcus Serum, C.c.								Serum and Antigen Controls
		0.001	0.0008	0.0006	0.0005	0.0004	0.0003	0.0002	0.0001	
Gonococcus.... 1:10	141†	++++	++++	++++	+++	++	+	—	—	—
	132B†	++++	++++	++++	+++	++	+	—	—	—
	132A‡	++++	++++	++++	+++	++	+	—	—	—
	250‡	++++	++++	++++	+++	++	+	—	—	—
		Amount of Antimeningococcus Serum, C.c.								
		0.001	0.0008	0.0006	0.0005	0.0004	0.0003	0.0002	0.0001	
Meningococcus 1:30	141†	++++	++++	++++	++++	+++	++	+	—	—
	132B†	++++	++++	++++	++++	+++	++	+	—	—
	132A‡	++++	++++	++++	++++	+++	++	+	—	—
	250‡	++++	++++	++++	++++	+++	++	+	—	—
		Amount of Antistreptococcus Serum, C.c.								
		0.01	0.008	0.006	0.005	0.004	0.003	0.002	0.001	
Streptococcus. 1:5	141†	++++	++++	++++	++++	+++	++	+	—	—
	132B†	++++	++++	++++	++++	+++	++	+	—	—
	132A‡	++++	++++	++++	++++	+++	++	+	—	—
	250‡	++++	++++	++++	++++	+++	++	+	—	—
		Amount of Antipollen Serum, C.c.								
		0.01	0.005	0.004	0.003	0.002	0.001	0.0009	0.0008	
Pollen..... 1:100	141†	++++	++++	++++	++++	+++	++	+	—	—
	132B†	++++	++++	++++	++++	+++	++	+	—	—
	132A‡	++++	++++	++++	++++	+++	++	+	—	—
	250‡	++++	++++	++++	++++	+++	++	+	—	—

* In this table, Citron's standard for the strength of a reaction is used: namely, complete absence of hemolysis is indicated by a 4 plus sign (++++); faint hemolysis is shown by a 3 plus sign (+++); partial hemolysis is shown by a 2 plus sign (++); nearly complete hemolysis is indicated by a single plus sign (+); while a minus sign (—) indicates complete hemolysis.

† These amboceptors were preserved in glycerol.

‡ No preservative was used in these amboceptors.

The serums from horses actively immunized against cultures of gonococci, meningococci, and streptococci were employed; also serum from rabbits immunized against pollen. Each of these serums was titrated against its homologous antigen.

As is shown in Table 2, glycerol in the amboceptor serum (141 and 132 B) did not interfere with the complement-fixation reaction in the slightest, the same degree of fixation resulting when a glycerolated amboceptor was used as when one without glycerol was used.

SUMMARY

1. Fresh antisheep hemolytic amboceptors that were heated to 55 C. for one-half hour, and then mixed with an equal volume of glycerol did not deteriorate, but retained their original titer for 3 years. During that period, anticomplementary properties did not develop.

2. The glycerol in the glycerolated antisheep hemolytic amboceptor did not influence the complement-fixation reaction.

3. Fresh antisheep hemolytic amboceptors that were inactivated and then preserved in glycerol, as herein described, were not only remarkably stable but were also protected from bacterial growth for a period of 3 years.